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Research Paper

Morphological and Molecular Analysis of *Trichuris* Species (Nematoda: Trichuridae) From *Capra Hircus* (L.) in Aurangabad District(M.S.) India.

Zoology

KEYWORDS : *Capra hircus*, India, *Trichuris*, *Trichuridae.*

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ABSTRACT

In the present work, a morphological and molecular analysis of Trichuris Roederer,1761 (Nematoda: Trichuridae) parasitizing Capra hircus has been carried out. The present nematode islarge in size,head is small, cuticle is transversely striated spicule single,Spicular shaft is funnel-shaped,testis convoluted.Vagina long with thick muscular wall, Uterus is elongated, long, curved, tail bluntly rounded.

In molecular analysis of present parasites, the genomic DNA were amplified and sequence. After both the molecular and morphological analysis the nematode is identified as confirmed to be representing genus Trichuris species in Capra hircus.

INTRODUCTION

The genus *Trichuris* established by Roederer 1761. The genus *Trichuris* are very common nematodes of the small ruminants. The *Trichuris* species are known to parasites of the intestine of goats in India. In the molecular analysis *Trichuris skarjabini* (Baskakaw,1924), *Trichuris discolor* (Linstow,1906) and *Trichuris leporis* (Froelich,1789) is closely related to (99%) of the present worm.

In the present study, we carried out morphological and molecular analysis of genus *Trichuris* from goats at Aurangabad (M.S.) India in the month of Feb. -2015.

MATERIAL AND METHODS

The nematode parasites were collected from the intestine of goat *Capra hircus (L.)* from Aurangabad during the period of June 2013 - May 2015.

Nematodes are collected from intestine of *Capra hircus* (L.) and preserved in hot 10% glycerol, cleared in lacto phenol, mounted in glycerin jelly and drawings are made with the aid of camera lucida. All measurements are given in millimeters. The identification is made with the help of Systema Helminthum.

Nematodes intended for molecular analysis were fixed with 95% ethyl alcohol. DNA Extraction was carried out using Genelute Mammalian Genomic DNA extraction kit (Sigma, G1N70-1KT). 25mg of tissue was minced and transferred to 1.5ml microcentrifuge tube. 180µl of Lysis solution T and 20 µl of proteinase K were added. The samples were mixed and incubated at 55^{0}C to digest the tissue completely. 20 µl of RNase A solution was added and incubated at room temperature for 2min. Then 200µl of lysis solution C was added and incubated at 70^{0}C for 10 min. The column was prepared for binding by adding 500µl of Column preparation solution to each pre-assembled GenElute Miniprep Binding Column and centrifuge at 12,000 rpm for 1 min. 200µl of ethanol was added to the lysate and mixed by vortexing. The entire lysate was transferred into the treated binding column and centrifuge at 10,000rpm for 1 min. The binding column was then placed in fresh 2ml collection tube. 500µl of Wash solution was added to the binding column and centrifuge at 10,000 rpm for 3min. This step was repeated twice. The column was again transferred to a new tube. 200µl of elution buffer was added directly into the centre of the binding column and centrifuge at 10,000rpm for 1min. Concentration of DNA was determined using UV-1800 spectrophotometer (Schimadzu Corporation A11454806498). The DNA was stored at -20^{0}C for further use.

The DNA isolated was subjected to polymerase chain reaction (PCR) amplification using Biometra thermal cycler (T-Personal 48). The PCR reaction mix contained 2.5µl of 10X buffer, 1µl of each primer (Table 6), 2.5µl of 2.5mM of each dNTP, 2.5 Units of Taq DNA polymerase and 1µl Template DNA and 8.5µl nuclease free water. The PCR amplification cycle consist of, a cycle of 5 min at 94°C; 35 cycles of 1min at 94°C, 1 min at 56°C, 2 min at 72°C; and additionally 1 cycle of 7 min at 72°C. The reagents used are procured from GeNei. Gel electrophoresis was performed using 1.0% agarose (Seakem, 50004L) to analyze the size of amplified PCR product. The band size obtained for amplification of Partial 18S rRNA region is ~1095bp.

The PCR product was purified using AxyPrep PCR Clean up kit (Axygen, AP-PCR-50). 100µl of PCR-A buffer was added to the 25µl of reaction. The sample was mixed and transferred to column placed in 2ml collection tube and centrifuge at 10,000 rpm for 1min. the filtrate was discarded. 700µl of W2 buffer was added to the column and centrifuge at 10,000rpm for 2min. This step was repeated twice. The column was transferred to a new tube. 25µl of Eluent was added into the column and incubated at room temperature for 2min. Then centrifuge at 10,000rpm for 5min. It was further sequenced using Applied Biosystems 3730xl DNA Analyzer USA and chromatogram was obtained. For sequencing of 18S rRNA PCR product 18s 5F- 5' (CTGGTTGATYCTGCCAGT 3') sequencing primer was used and for sequencing 28S rRNA PCR product LSU5F 5' (TAGGTCGACCCGCTGAAYTTAAGCA) sequencing primer was used.

The DNA sequences were analyzed using online BLASTn (nucleotide Basic Local Alignment Search Tool) facility of National Centre for Biotechnology Information (NCBI). The BLAST results were used to find out evolutionary relationship of Worms. Altogether twenty sequences, including sample were used to generate phylogenetic tree (Figure -3). The tree was constructed by using MEGA 5 software (Saitou N. and Nei M.,1987; Felsenstein J.1985 and Tamura K. et al 2011).

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RESULTS

Morphological analysis: - The nematode parasites is fairly long in size, elongated, unsegmented, creamy white in colour. The head is very small, cuticle is transversely striated, a weakly developed "bacillary band" is also present, the mouth leads in to capillary oesophagus and posterior portion of oesophagus is expanded.

Male: - Male is smaller than female**.** Anterior part of the body is thin, long and measure 6.65mm in length and 0.305mm (0.076-0.534) in width. Middle part of the body measure 14.63mm in length and 2.365mm (1.678-3.052) in width. Posterior part of the body is thick, curved in shape and measure 31.35mm in length and 1.237mm (1.188-1.287) in width. The oesophagus measuring 6.65mm in length and 0.133mm (0.076-0.19) in width. In male, spicule single and 10.67mm in length and 0.099mm (0.033-0.165) in width.cirrus is present above or anterior to spicule. Cloacal length is measures 7.86mm in length. Spicular shaft is funnel-shaped. Anterior end of the body, vas deference and ejaculatory duct is present.

Female : -Female is longer than male. Anterior part of the body is thin, long in size and measure 12.99mm in length and 0.629mm (0.457-0.801) in width. Middle part of the body measure 7.67mm in length and 1.831mm (1.144-2.518) in width. . Posterior part of the body is thick, blunt and measure 14.21mm in length and 1.506mm (0.228-2.785) in width. The oesophagus measuring 12.99 mm in length and 0.552 mm (0.457-0.648) in width. Vulva measures 3.81 mm in length and 0.095 mm (0.076-0.114) in width. Uterus is elongated, long, curved and full filled of the eggs.

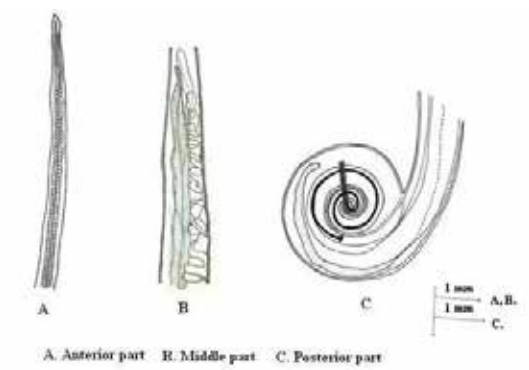


Fig. 1. Male. ***Trichuris ovis*** **, Abildgaard, 1795**

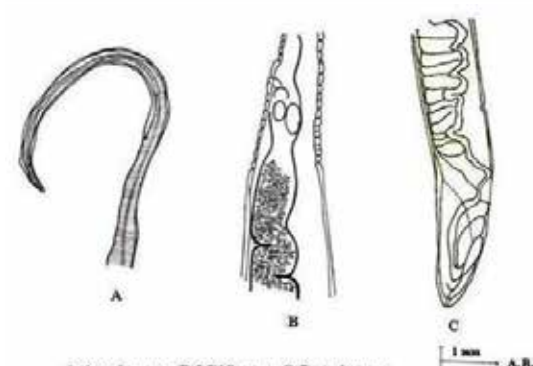


Fig. 2. Female. ***Trichuris ovis*****, Abildgaard, 1795**

Molecular analysis:- Partial 18s rRNA gene Sequence (1017bp) Sequence length: 1017bp

Table 1: Phylogenetic neighbors of sample based on partial 18s rRNA gene sequence

Description	Max score		E val-ue	Ident	Accession
Trichuris skrjabini 18S rRNA gene, strain Tsk1	1731	92%	0.0	99%	HF586912.1
Trichuris leporis 18S rRNA gene, strain Tl1	1714	92%	0.0	99%	HF586913.1
Trichuris ovis 18S rRNA gene, strain To1	1694	92%	0.0	99%	HF586911.1
Trichuris discolor 18S rRNA gene, strain Td1	1688	92%	0.0	99%	HF586910.1
Trichuris arvicolae 18S rRNA gene, strain Ta1	1526	92%	0.0	96%	HF586908.1
Trichuris muris 18S rRNA gene, strain Tm1	1526	92%	0.0	96%	HF586907.1
Trichuris suis isolate Ovamed 18S ribosomal RNA gene, partial sequence	1517	93%	0.0	95%	EU790668.1
Trichuris suis strain TriUSui1 small subunit ribosomal RNA gene, partial sequence	1511	93%	0.0	95%	FJ969143.1
Trichuris suis 18S ribosomal RNA gene, partial sequence	1511	93%	0.0	95%	AY856093.1
Trichuris vulpis 18S rRNA gene, strain Tv1	1509	92%	0.0	96%	HF586909.1
Trichuris suis 18S rRNA gene, strain TS1	1504	92%	0.0	95%	HF586905.1
Trichuris sp. CC-2013 18S rRNA gene, strain Tsp1	1487	92%	0.0	95%	HF586906.1
Trichuris suis 18S ribosomal RNA gene, complete sequence	1485	93%	0.0	95%	AY851265.1
Trichuris vulpis isolate D56 clone C 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S ribosomal RNA gene, partial sequence	1461	90%	0.0	95%	GQ352558.1
Trichuris vulpis isolate D56 clone B 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S ribosomal RNA gene, partial sequence	1461	90%	0.0	95%	GQ352557.1
Trichuris vulpis isolate D56 clone A 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S ribosomal RNA gene, partial sequence	1456	90%	0.0	95%	GQ352556.1
Trichuris muris 18S ribosomal RNA gene, partial sequence	1417	90%	0.0	94%	AF036637.1
Trichuris trichiura small subunit ribosomal RNA gene, partial sequence	1406	93%	0.0	93%	DQ118536.1
Trichuris trichiura isolate TH2 clone E 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S ribosomal RNA gene, partial sequence	1384	90%	0.0	94%	GQ352551.1
Trichuris trichiura isolate TH2 clone B 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S ribosomal RNA gene, partial sequence	1384	90%	0.0	94%	GQ352548.1

Figure 3: Phylogenetic tree for sample using partial 18s rRNA gene sequence

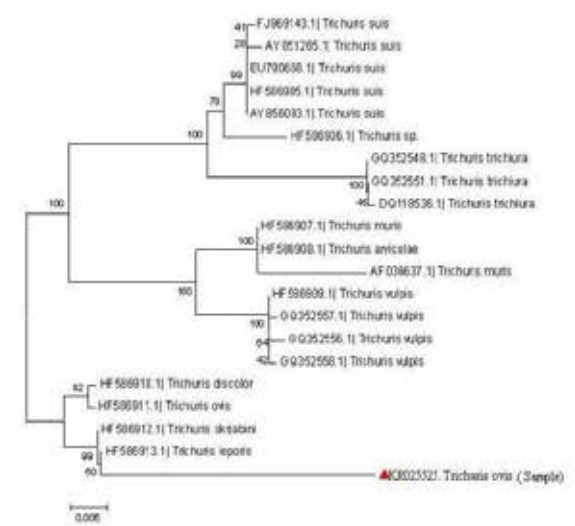
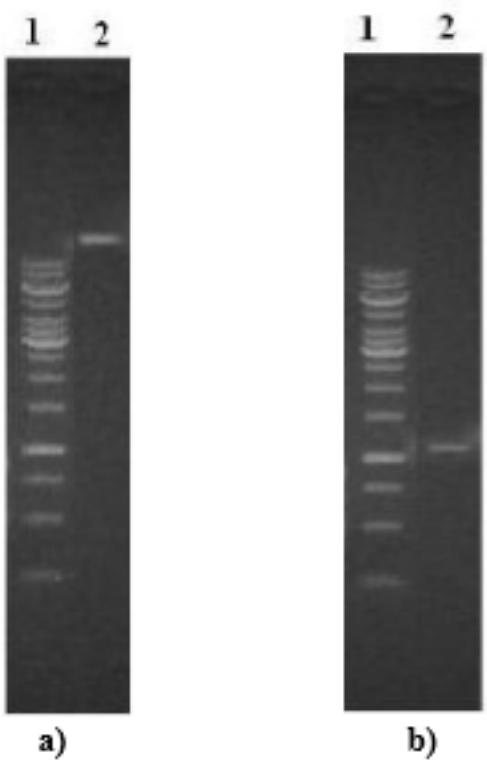


Figure 4: a) Genomic DNA extracted from worms
b) Amplification of partial 18S and 28S rRNA gene for worm samples



Lane 1 a: 1Kb DNA Ladder of Fermentas

Lane 1 b) : 1Kb DNA Ladder of Fermentas

Lane 2a: Genomic DNA of wormsLane 2b: Am

plified PCR product of ***Trichuris*** **species(sample)**

1Kb DNA marker (Top to bottom): 10000, 8000, 6000, 5000, 4000, 3500, 3000, 2500, 2000, 1500, 1000, 750, 500 and 250bp.

Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura-Nei method and are in the units of the number of base substitutions per site. The analysis involved 21 nucleotide sequences.

DISCUSSION

After going through the literature the present nematode parasite resembles *Trichuris Ovis* (Abildgaard, 1795) in having all the essential morphological character i,e body is fairly long in size and creamy white in colour. The head is very small, cuticle is transversely striated, a weakly developed "bacillary band" is also present, tail is blunt, curved. Spicular shaft is funnel-shaped, spicule sheath is tubular in shape covered with small spines. Spicule single and long,testes begins at the proximal end of the cloaca, testis convoluted in vas deferens regions and tightly coiled as it proceeds towards anterior end of the body, oesophagus long, vas deference and ejaculatory duct is present, Uterus is elongated, long, curved and filled of the eggs. Vagina long with thick muscular wall, tail bluntly rounded and reported from *capra hircus (L.)* in india.

We conclude that the morphological observation as well as the sequence of its 18S rRNA gene obtained in this study, clearly demonstrate that this species should be considered to be a member of genus *Trichuris* (Nematoda: Trichuridae) but the species having similar with *Trichuris ovis* (99%) in both the observation that is morphological and molecular analysis. It also identified closed with other species of *Trichuris* that is *Trichuris discolor*, (Von Linstow, 1906) (99%) *Trichuris leporis* (Froelich,1789) (99%) and *T. skrjabini* (Baskakov,1924) (99%) from comparison with *T. Ovis* (Abildgaard,1795) (Table no. 1).

In molecular analysis the phylogenetic neighbors of *Trichuris* Sp. based on partial 18S rRNA gene are shown in table no. 1. On the basis of position of sequence of the given *Trichuris* sample in the phylogenetic tree, the sample showed closest similarity with the of *Trichuris ovis* (99%).

After discussion both morphological and molecular observations the character comes closer to *Trichuris Ovis,* hence it is redescribed here as *Trichuris Ovis* (Abildgaard, 1795) (99%).

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